

AMENDMENTS

Amendment to the Claims:

Please amend the claims as follows:

This listing of claims will replace all prior versions and listing of the claims in the application:

Listing of the Claims:

Claim 1 (currently amended): An isolated, a synthetic, or a recombinant nucleic acid comprising:

(a) a nucleic acid sequence having at least 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:7, wherein the nucleic acid encodes a polypeptide having glucoamylase activity;

(b) a nucleic acid sequence encoding a polypeptide at least 95% identical to SEQ ID NO:8, wherein the polypeptide has glucoamylase activity;

(c) a nucleic acid sequence encoding a fragment of the polypeptide of (a) or (b), wherein the fragment has at least 95% sequence identity to a sequence as set forth in SEQ ID NO:7 or encodes a polypeptide having at least 95% sequence identity to a sequence as set forth in SEQ ID NO:8, and wherein the fragment encodes a polypeptide having glucoamylase activity;

(d) the nucleic acid of any of (a) to (c), wherein the nucleic acid encodes the polypeptide lacking a signal (leader) sequence; or

(e) a nucleic acid sequence fully complementary to any of (a) to (d).

Claims 2 to 45 (canceled)

Claim 46 (currently amended): A nucleic acid probe for identifying a nucleic acid encoding a polypeptide with glucoamylase activity, wherein the probe comprises at least 70 consecutive bases of the nucleic acid sequence of claim 1; wherein the probe identifies the nucleic acid by hybridization under high stringency conditions, wherein the hybridization

conditions include a wash step comprising a wash in ~~[[0.2X]]~~ 0.1X SSC containing 0.1% SDS at a temperature of about ~~[[65°C]]~~ 68°C for about 15 minutes, and the identified nucleic acid sequence has at least 95% sequence identity to SEQ ID NO:7, and the identified nucleic acid encodes a polypeptide having a glucoamylase activity.

Claims 47 to 55 (canceled)

Claim 56 (previously presented): An expression cassette comprising the nucleic acid sequence of claim 1.

Claim 57 (previously presented): A vector comprising the nucleic acid sequence of claim 1.

Claim 58 (previously presented): A cloning vehicle comprising the nucleic acid of claim 1, or the vector as set forth in claim 57.

Claims 59 to 60 (canceled)

Claim 61 (previously presented): An isolated cell comprising the vector of claim 57, or the nucleic acid of claim 1.

Claims 62 to 132 (canceled)

Claim 133 (previously presented): An array comprising an immobilized nucleic acid comprising the nucleic acid of claim 1.

Claims 134 to 168 (canceled)

Claim 169 (currently amended): A method for isolating or recovering a nucleic acid encoding a polypeptide with a glucoamylase activity from an environmental sample comprising:

- (A) ~~[[a)]~~ (i) providing a polynucleotide probe comprising the nucleic acid sequence of claim 1, or a subsequence thereof;
~~[[b)]~~ (ii) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to a polynucleotide probe of step ~~[[a)]~~ (i);
~~[[c)]~~ (iii) combining the isolated nucleic acid or the treated environmental sample of step ~~[[b)]~~ (ii) with the polynucleotide probe of step ~~[[a)]~~ (i); and
~~[[d)]~~ (iv) isolating a nucleic acid that specifically hybridizes with the polynucleotide probe of step ~~[[a)]~~ (i), wherein the nucleic acid has at least 95% sequence identity to SEQ ID NO:7 or encodes a polypeptide having at least 95% sequence identity to SEQ ID NO:8, and wherein the nucleic acid encodes a polypeptide having glucoamylase activity, thereby isolating or recovering a nucleic acid encoding a polypeptide with a glucoamylase activity from an environmental sample, or
- (B) the method of (A), wherein the environmental sample comprises a water sample, a liquid sample, a soil sample, an air sample or a biological sample, or
- (C) the method of (B), wherein the biological sample is derived from a bacterial cell, a protozoan cell, an insect cell, a yeast cell, a plant cell, a fungal cell or a mammalian cell.

Claim 170 (canceled)

Claim 171 (currently amended): A method of generating a variant of a nucleic acid encoding a polypeptide with a glucoamylase activity comprising:

- (A) ~~[[a)]~~ (i) providing a template nucleic acid comprising the nucleic acid sequence of claim 1; and
~~[[b)]~~ (ii) modifying, deleting or adding one or more nucleotides in the template sequence, or a combination thereof, to generate a variant of the template nucleic acid, wherein the variant nucleic acid has at least 95% sequence identity to SEQ ID NO:7 or encodes a polypeptide having at least 95% sequence identity to SEQ ID NO:8, and wherein the variant nucleic acid encodes a polypeptide having glucoamylase activity, or

(B) the method of (A), wherein the method further comprises expressing the variant nucleic acid to generate a variant glucoamylase polypeptide, or the modifications, additions or deletions are introduced by a method comprising error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturation mutagenesis (GSSM), synthetic ligation reassembly (SLR), recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and a combination thereof, or

(C) the method of (A), wherein the method is iteratively repeated until a glucoamylase having an altered or different activity or an altered or different stability from that of a polypeptide encoded by the template nucleic acid is produced, or

(D) the method of (C), wherein the variant glucoamylase polypeptide is thermotolerant and retains some activity after being exposed to an elevated temperature, or the variant glucoamylase polypeptide has increased glycosylation as compared to the glucoamylase encoded by a template nucleic acid, or

(E) the method of (C), wherein the variant glucoamylase polypeptide has a glucoamylase activity under a high temperature, wherein the glucoamylase encoded by the template nucleic acid is not active under the high temperature, or

(F) the method of (C), wherein the method is iteratively repeated until a glucoamylase coding sequence having an altered codon usage from that of the template nucleic acid is produced, or

(G) the method of (C), wherein the method is iteratively repeated until a glucoamylase gene having higher or lower level of message expression or stability from that of the template nucleic acid is produced.

Claims 172 to 274 (canceled)

Claim 275 (currently amended) An isolated, a synthetic or a recombinant nucleic acid sequence that hybridizes under stringent conditions to the full-length complement of a nucleic acid comprising the sequence of SEQ ID NO:7,

wherein the stringent conditions include a wash step comprising a wash in ~~[[0.2X]]~~ 0.1X SSC containing 0.1% SDS at a temperature of about ~~[[65°C]]~~ 68°C for about 15 minutes, and the nucleic acid sequence has at least 95% sequence identity to SEQ ID NO:7, and the nucleic acid encodes a polypeptide having a glucoamylase activity.